ACUTE CORONARY SYNDROMES

One-day kinetics of myocardial engraftment after intracoronary injection of bone marrow mononuclear cells in patients with acute and chronic myocardial infarction

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Objective: To investigate the kinetics of myocardial engraftment of bone marrow-derived mononuclear cells (BMNCs) after intracoronary injection using ^{99m}Tc-d,l-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) nuclear imaging in patients with acute and chronic anterior myocardial infarction.
Design: Nuclear imaging-derived tracking of BMNCs at 2 and 20 h after injection in the left anterior descending (LAD) coronary artery.
Setting: Academical cardiocentre.
Patients: Five patients with acute (mean (SD) age 58 (11) years; ejection fraction range 33–45%) and five patients with chronic (mean (SD) age 50 (6) years; ejection fraction range 28–34%) anterior myocardial infarction.
Interventions: A total of 24.2×10^8–57.0×10^8 BMNCs (20% labelled with 700–1000 MBq ^{99m}Tc-HMPAO) were injected in the LAD coronary artery.
Results: At 2 h after BMNC injection, myocardial activity was observed in all patients with acute (range 1.31–5.10%) and in all but one patient with chronic infarction (range 1.10–3.0%). At 20 h, myocardial engraftment was noted only in three patients with acute myocardial infarction, whereas no myocardial activity was noted in any patient with chronic infarction.
Conclusions: Engraftment of BMNCs shows dynamic changes within the first 20 h after intracoronary injection. Persistent myocardial engraftment was noted only in a subset of patients with acute myocardial infarction.

Intracoronary administration of autologous bone marrow-derived mononuclear cells (BMNCs) is emerging as an adjunctive therapy to promote regeneration of infarcted myocardium.[1] Nevertheless, the efficacy of intracoronary injection to deliver BMNCs into the diseased human myocardium remains controversial. Experimental studies using radioactive labelling and/or immunohistochemical analysis revealed that fewer than 3% of transferred cells engraft in the heart after injection into the left ventricular (LV) cavity[2–4] or after intracoronary injection.[5]

Radioisotope cell labelling with ^{99m}Tc-d,l-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) is a well-established clinical method to monitor the distribution of systemic cells.[6] ^{99m}Tc-HMPAO forms a lipid-soluble neutral complex, which can readily penetrate cellular membranes and is rapidly incorporated into cells. The 6 h physical half-life of ^{99m}Tc allows reliable tracking of transferred cells for 24 h.[7]

Therefore, the aim of the present study was to investigate the 1-day kinetics of myocardial engraftment of autologous BMNCs after intracoronary injection using ^{99m}Tc-HMPAO nuclear imaging in patients with acute and chronic anterior myocardial infarction.

METHODS

Patients

The study population consisted of two groups. The first group comprised patients who had their first ST-segment elevation acute anterior myocardial infarction (n = 5, mean (SD) age 58 (11) years; 100% males) due to occlusion of the proximal left anterior descending (LAD) coronary artery treated by primary stented angioplasty. Other inclusion criteria included complete restoration of the Thrombolysis in Myocardial Infarction 3 flow in the infarct-related artery and reduced LV ejection fraction (LVEF) $\leqslant$45% with akinesia of the LAD coronary artery perfusion territory on echocardiogram performed 2 days after the intervention. Time from onset of pain to reperfusion ranged from 4 to 12 h. Patients with haemodynamic instability (Killip III, IV) and multivessel coronary artery disease were excluded. The second group comprised patients with chronic ischaemic heart failure (New York Heart Association III; n = 5, mean (SD) age 50 (6) years; 80% males). Inclusion criteria were: (1) history of anterior myocardial infarction >6 months treated with stented angioplasty in the affected LAD coronary artery; (2) patent LAD coronary artery and no in-stent restenosis on index coronary angiography; (3) stable LV dysfunction (LVEF <35% for at least 6 months) on echocardiography. Patients with recent acute coronary syndrome, multivessel disease or recent revascularisation ($\leqslant$6 months) were excluded. The study protocol was approved by the medical ethical committees of the Charles University, Prague, Czech Republic, and informed consent was obtained from all patients.

BMNC isolation and ^{99m}Tc-HMPAO labelling

BMNC aspiration was performed 3–10 days after index-stented angioplasty in patients with acute myocardial infarction, or electively in patients with chronic heart failure. Bone marrow blood (200–250 ml) was aspirated under analgosedation from both iliac crests. Mononuclear BMNCs were isolated using Ficoll density gradient centrifugation and resuspended in a

Abbreviations: BMNC, bone marrow-derived mononuclear cell; CK, creatine kinase; FDG, [^{18}F]-fluoro-2-deoxy-D-glucose; LAD, left anterior descending; LV, left ventricular; LVEF, left ventricular ejection fraction; ^{99m}Tc-HMPAO, ^{99m}Tc-d,l-hexamethylpropylene amine oxime

final volume of 25–35 ml. In all, 20% (5–7 ml) of the cell suspension was separated and labelled with 700–1000 MBq ^{99m}Tc-HMPAO according to the manufacturer's instructions (Medi-Radiopharma, Budapest, Hungary). Supernatant and cell-bound radioactivity was measured in a calibrated well counter. Labelling efficiency was calculated as cell-bound radioactivity/(cell-bound radioactivity+supernatant radioactivity) immediately after the labelling procedure. ^{99m}Tc-HMPAO retention in BMNCs over time (labelling stability) was calculated as the labelling efficiency at 1, 2 and 20 h after labelling. BMNC viability was assessed by the trypan blue exclusion test. The proliferative capacity of cells was tested using MethoCult GF H4434 assay (StemCell Technologies, Vancouver, Canada).[8]

BMNC intracoronary injections

Before injection, radiolabelled BMNCs were mixed back with non-labelled cell suspension. The whole volume of cell suspension (25–35 ml) was injected in 5–7 separate injections, each of them containing 4–6 ml of BMNC suspension. In all patients, BMNC suspension was injected into the LAD coronary artery through the central lumen of an over-the-wire balloon catheter, as described previously.[1] Briefly, low-pressure balloon inflations within the stented segment were performed up to 3 min or up to the maximally tolerated time, and followed by 3 min reperfusion. This procedure was repeated 5–7 times, depending on the initial volume of BMNC suspension. Cardiac troponin I as well as creatine kinase (CK) and its isoenzyme (CK-MB) were assessed before and serially after BMNC transfer.

Nuclear imaging

Whole-body images, spot views of the liver and urinary bladder, and single photon emission CT images of the thorax were taken at 2 and 20 h after the BMNC injection with a dual-head γ camera (Helix, Elscint, Haifa, Israel) using a low-energy, high-resolution collimator. Whole-body images were acquired in the anterior and posterior views and stored in 256×1024 matrix. Spot views were acquired using overflow correction in a 256×256 matrix. Single photon emission CT images (30 images, 30 s/image, 64×64 matrix) were obtained over 180° (from the 45° right anterior oblique to the 45° left posterior oblique projection), using a 10% symmetric energy window centred at 140 keV.

Statistical analysis

Data are presented as mean (SD).

RESULTS

There were no complications during BMNC aspiration in any patient. After Ficoll isolation, the total number of BMNCs ranged from 24.2×10^8 to 57.0×10^8 (CD34 0.39–0.72%). BMNC suspension was injected in the LAD coronary artery in all patients. Thrombolysis in Myocardial Infarction 3 flow in the LAD coronary artery, both the before and after PCI, was present in all patients. The mean (SD) total time of LAD coronary artery occlusion was similar in both groups of patients (1151 (152) s vs 1090 (201) s, p = NS). No periprocedural complications or increase in cardiac markers were observed after BMNC intracoronary injections. In patients with acute myocardial infarction, the mean (SD) values for CK before and after BMNC injection were 451 (183) and 437 (159) U/l (p = NS), respectively, and those for troponin I 251 (196) μg/l and 260 (181) (p = NS), respectively. In patients with chronic myocardial infarction, the mean (SD) values for CK before and after BMNC injection were 55 (21) and 49 (30) U/l (p = NS), respectively, and those for troponin I were undetectable (<0.5 μg/l).

BMNC labelling with ^{99m}Tc-HMPAO

The mean (range) labelling efficiency of BMNCs with ^{99m}Tc-HMPAO was 90.05% (88.62–91.48%). The mean (SD) percentage of radioactivity released from BMNCs over time was 4.16 (2.32), 6.04 (3.01), and 13.62 (5.20) at 1, 2 and 20 h, respectively, after labelling. The viability of radiolabelled BMNCs was comparable to that of non-labelled BMNCs (range 94–99%) and their ability to proliferate was not altered.

Myocardial engraftment and dynamics of ^{99m}Tc-HMPAO-labelled BMNCs in patients with acute myocardial infarction

No patients showed signs of microvascular obstruction immediately before BMNC transfer. Figure 1 shows the body distribution of BMNCs in patient with an acute myocardial infarction and transient myocardial engraftment. Figure 2 shows the body distribution of BMNCs in patient with an acute myocardial infarction and persistent myocardial engraftment. Table 1 shows individual data of BMNCs processing, intracoronary transfer and myocardial uptake.

At 2 h after BMNC injection, myocardial activity was observed in all patients and myocardial uptake ranged from 1.31% to 5.10%. At 20 h after injection, the myocardium-associated activity (1.00%–1.34%) was detected only in three patients, and was absent in the remaining two patients (patients 2 and 4). In all patients, the myocardium-bound activity was confined to the LAD coronary artery perfusion territory. Although at 2 h BMNCs occupied the whole LAD coronary artery territory, at 20 h engraftment was confined to more distal parts of the infarction territory (figs 1 and 2). At 4-month follow-up, LVEF increased significantly as compared with that at baseline (40% (5.1%) vs 45% (6.2%), p = 0.02). The magnitude of improvement in ejection fraction was related neither to the number of infused cells nor to the percentage of engrafted cells.

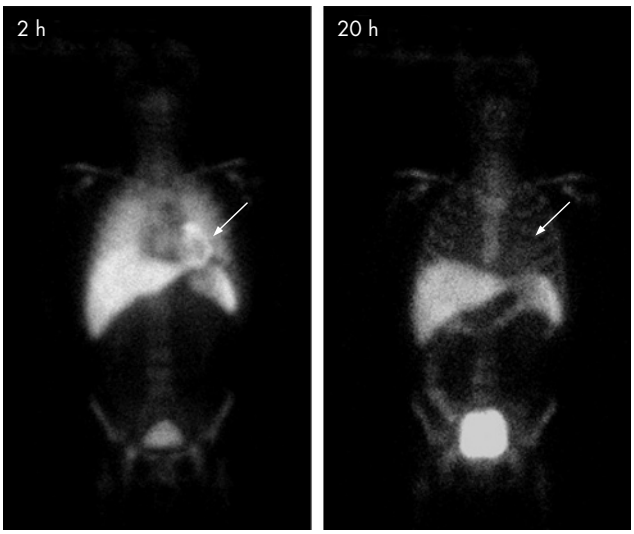

Figure 1 An example of organ distribution of ^{99m}Tc-d,l-hexamethylpropylene amine oxime-labelled bone marrow-derived mononuclear cells (BMNCs) in a patient with an acute anterior myocardial infarction and transient myocardial engraftment. BMNCs were injected in the left anterior descending (LAD) coronary artery. Two-hour whole body scans (left panel) showed large pools of activity in the spleen, liver and lungs. No activity was observed in the brain. Nevertheless, labelled BMNCs were also detected in the majority of the LAD coronary artery-supplied myocardium (arrow). At 20-h scans (right panel), significant activity remained in the spleen, liver and urinary bladder. However, myocardial engraftment was no longer observed, suggesting transient myocardial retention in this patient.

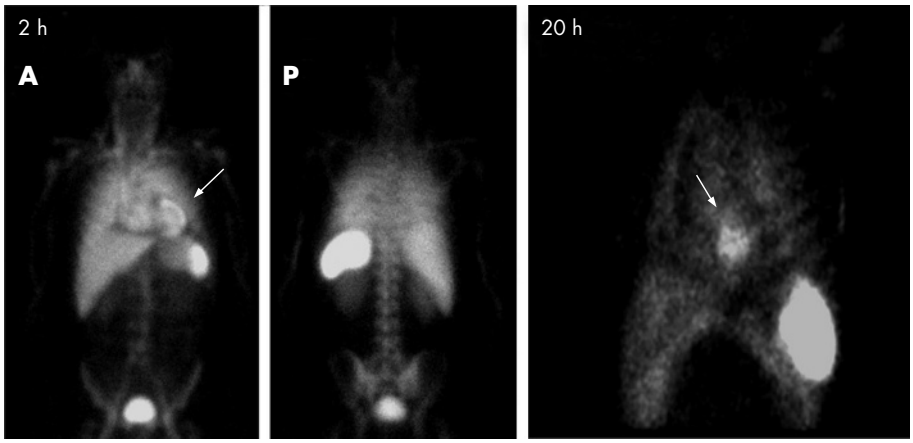

Figure 2 An example of organ distribution of ^{99m}Tc-d,l-hexamethylpropylene amine oxime-labelled bone marrow-derived mononuclear cells in a patient with an acute anterior myocardial infarction and persistent myocardial engraftment. At 2 h (left panel), whole body anterior (A) and posterior (P) scans showed similar organ distribution as in the patient described in the legend to fig 1 with the presence of significant activity in the circulating blood, spleen, liver, lungs and urinary bladder. Activity uptake by the heart was 4.50%, and myocardial homing was distributed in the whole left anterior descending coronary artery territory (arrow). At 20 h (right panel; the left anterior oblique thorax planar spot view), persistent myocardial engraftment (1.34%) was observed only in the apical region of the left ventricle (arrow), at the site of infarct and border zones. The majority of transplanted cells were located in spleen.

Myocardial engraftment and dynamics of ^{99m}Tc-HMPAO-labelled BMNCs in patients with chronic ischaemic heart failure

Figure 3 shows the biodistribution of BMNCs in a patient with chronic ischaemic heart failure. At 2 h after injection, activity in the heart could be detected in all but one patient (table 1). In contrast to patients with acute myocardial infarction, the distribution of signal was diffuse and not solely restricted to the LAD coronary artery perfusion territory (fig 3). In addition, no activity remained in the myocardium 20 h after injection in any patient. The baseline and 4-month follow-up LVEFs were similar (31% (2.6%) vs 31% (1.9%), p = NS).

In all patients, the majority of injected activity (>80%) was detected in the spleen, liver and lungs. No BMNCs engrafted in the brain. No association could be demonstrated between myocardial engraftment and age, risk factors including diabetes mellitus and statin use.

DISCUSSION

This study investigates the fate of ^{99m}Tc-HMPAO-labelled BMNCs after selective coronary injections in patients with acute myocardial infarction and chronic ischaemic heart failure. The biodistribution and myocardial engraftment of BMNCs were monitored for 20 h, which is the longest time period reported so far in humans. The main findings are: (1) at 2 h after BMNC injections, myocardial activity was observed in all patients with acute myocardial infarction and in all but one patient with chronic ischaemic heart failure, and (2) at 20 h after BMNC injections, persistent myocardial engraftment was noted only in three patients with acute myocardial infarction, whereas none was noted in patients with chronic ischaemic heart failure.

Transient or persistent engraftment of BMNCs

The concept of intracoronary stem cell therapy is highly attractive to promote cardiac regeneration of injured myocardium. It is based on the assumption of cell engraftment at the infarction site during the transcoronary passage.[4 9] Trafficking of BMNCs to the infarcted myocardium is regulated by chemokines and adhesion molecules induced by ischaemic injury.[10 11] Stromal cell-derived factor 1 seems to be the key player for homing of BMNCs to the myocardium.[12–14] However, data available to firmly establish distribution and engraftment rates using this approach are limited. Three experimental studies monitored myocardial homing of BMNCs after injection in the LV cavity using immunohistochemical analysis at different time points.[2–4] In a murine model of intact heart, dispersed engraftment of individual cells, as low as 0.44%, throughout the entire myocardium was detected at 4–60 days.[2] In a rat model of acute myocardial infarction, rates of engraftment ranged between 1% and 3% after intracavitary injections.[3 4] Of note, the myocardial homing was confined to the

Table 1 Individual characteristics

Patient	Age (years)	Risk factors	Time from symptom onset to PCI (h)/ history of MI (years)	Total BMNCs injected ($\times 10^8$)	LVEF before BMNCs transfer (%)*	LVEF after BMNCs transfer (%)*	Myocardial uptake of labelled BMNCs (% of injected activity)	
							2 h after injection	20 h after injection
Acute MI								
1	58	H	10/NA	35.40	40	43	5.10	1.10
2	67	DM, HL	4/NA	33.02	45	51	1.31	0
3	45	DM, H, HL	8/NA	37.68	33	35	2.10	1.00
4	50	H	5/NA	29.50	44	49	1.47	0
5	72	H, HL	5/NA	24.20	36	45	4.50	1.34
Chronic MI								
6	50		NA/5	46.75	29	31	1.60	0
7	51	H, HL	NA/3.5	57.00	28	30	0	0
8	41	H, HL	NA/2	46.00	34	31	3.00	0
9	49	H, HL	NA/4.5	44.24	33	29	1.10	0
10	57	DM, H, HL	NA/3.3	39.60	30	34	2.10	0

BMNC, bone marrow-derived mononuclear cell; DM, diabetes mellitus; H, hypertension; HL, hyperlipidaemia; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NA, not applicable; PCI, percutaneous coronary intervention.
*Baseline and follow-up LVEF were assessed by left ventricular angiography immediately before and 4 months after BMNC injections.

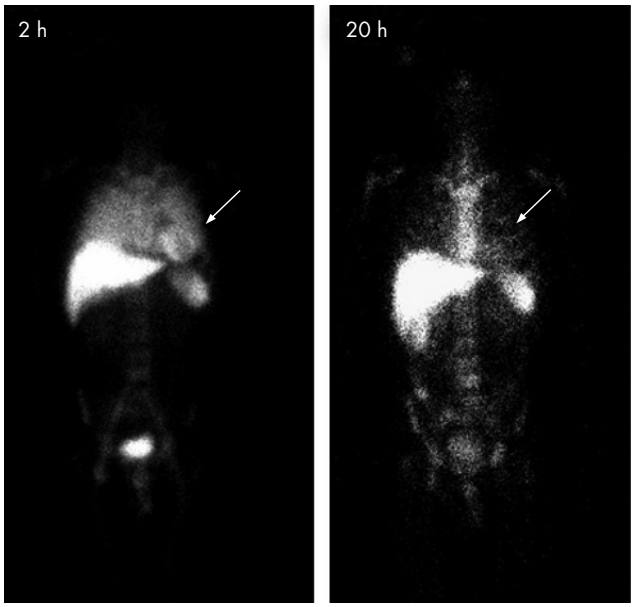

Figure 3 An example of organ distribution of ^{99m}Tc-d,l-hexamethyl-propylene amine oxime-labelled bone marrow-derived mononuclear cells (BMNCs) in a patient with chronic anterior infraction. At 2 h (left panel), the myocardial signal (arrow) of labelled BMNCs was detected not only in the anterolateral but also in the inferior left ventricular (LV) wall. At 20 h (right panel), no activity was observed in the heart in any LV segments. Both 2 and 20 h scans showed large pools of activity in the spleen and liver.

site of infarct and its border zone, and, in contrast to the intact murine heart,[2] no donor cells were detected in the remote myocardium. In a pig model, homing of ^{111}indium-oxine-labelled human peripheral blood mononuclear cells was assessed after selective infusion in the LAD coronary artery using γ-emission counting of harvested organs 4 h after injection. Though low activity of approximately 3% was detected in the infarcted anterolateral and apical regions of the left ventricle, the majority of transplanted cells were entrapped in the lungs.

In humans, Hofmann *et al*[15] studied immediate biodistribution of BMNCs after intracoronary injections in patients with recent acute myocardial infarction using [^{18}F]-fluoro-2-deoxy-D-glucose (FDG) labelling at positron emission tomography. They showed that 1.3–5.3% of unselected BMNCs (n = 6 patients) and 14–39% of selected CD34 cells (n = 3) homed to the myocardium 50–75 min after intracoronary injection. Although the majority of cells homed to the spleen, liver or lungs, labelled cells were consistently found in the area of the culprit vessel at the infarct centre and border zone. This study suggested that CD34-enriched cells have higher retention in infarcted myocardium than a mixture of unselected BMNCs. However, this finding was not confirmed by Blocklet,[16] who showed only 5.5% myocardial retention of FDG-labelled CD34-enriched cells 1 h after intracoronary injection. Corroborating the findings of Hofmann *et al*,[15] immediate myocardial retention of unselected BMNCs in our study ranged between 1.31% and 5.10% 2 h after the injection. A novel finding of the present study indicates that engraftment of BMNCs after intracoronary injection is dynamic already within the first 24 h. In fact, at 20 h, residual myocardial activity was detected only in three patients, whereas it was no longer detected in the remaining two patients. In addition, for the first time, the current study provides data on homing of BMNCs in patients with chronic ischaemic heart failure. In these patients, intracoronary injection of labelled BMNCs was associated only with transient diffuse myocardial activity throughout the whole myocardium immediately after injection, disappearing later at 20 h.

Mechanistic implications

These findings have several mechanistic implications for design and interpretation of ongoing clinical stem cell studies. First, myocardium-associated activity observed immediately after intracoronary injection seems to reflect transient cell retention not necessarily corresponding to the number of truly engrafted BMNCs. In contrast, myocardium-associated activity observed in a later time interval reflects more likely myocardial engraftment relevant for the functional outcome. The factors and mechanisms accounting for transient cell retention are possibly related to the adhesive status of the microcirculatory compartment or to the functional features of BMNCs. This needs to be addressed in future clinical and experimental studies using serological or tissue cytokine profiling. In addition, further studies are needed to investigate whether pharmacological modulation of microcirculation at the time of cell injection could enhance cell homing and abolish its transient nature. Given the controversial differentiation potential of haematopoietic stem cells,[17] one may also argue that the 1.00–1.34% engraftment seen in the infarcted myocardium is too small to exert functional effects. In the present study, the magnitude of improvement in ejection fraction was related neither to the number of infused cells nor to the percentages of engrafted cells, suggesting that the paracrine effect of transplanted cells could be the major underlying mechanisms for functional benefit after BMNC delivery. Finally, it is interesting to note the differences in the acute homing of injected cells between patients with acute and those with chronic myocardial infarction. Although the clinical study cannot provide adequate mechanistic explanation, it could be hypothesised that, similar to the diffuse homing observed in intact murine hearts,[2] the transient and diffuse character of the homing could be related to the absence of ischaemia-driven homing stimuli. It should also be noted that current experimental knowledge of homing signals pertains to the early time window after the myocardial infarction,[18] and that further studies are needed to explain the paradox between the absence of persistent homing and the functional effects observed in the early clinical studies.[19]

BMNC labelling with ^{99m}Tc-HMPAO

Labelling of leucocytes with ^{99m}Tc is widely used to detect sites of inflammation in clinical medicine.[6] In this study, we showed excellent labelling efficiency and very high retention of ^{99m}Tc-HMPAO within the BMNCs even 20 h after labelling. Hence, significant extravasation of ^{99m}Tc-HMPAO out of BMNCs could be excluded, and most of the activity observed on the scans attributed to BMNCs.

Limitations

Although, similar to previous studies,[3 20] the viability of ^{99m}Tc-HMPAO-labelled BMNCs was not altered, the adverse effects of labelling on the migratory and functional abilities of BMNCs cannot be entirely excluded. Botti *et al*[20] compared the effect of the toxicity of three commonly used labelling agents on activated human T lymphocytes. Both indium-111 oxine and FDG impaired the cytotoxic activity of labelled lymphocytes, whereas no adverse functional effects were induced by ^{99m}Tc-HMPAO. Nevertheless, all three agents reduced the proliferative ability of labelled cells significantly. In that study, myocardial uptake of only unfractionated mononuclear bone marrow cells was investigated. No enriched subpopulations, such as CD34 or ACC133 cells, were studied specifically. The engraftment of these selected cells may be higher than that of unselected BMNCs[15] because of less competition for endothelial binding sites in the target coronary artery.

CONCLUSIONS

Cell homing of labelled BMNCs seems to be dynamic in the first 20 h after intracoronary injections, resulting in only transient myocardial activity in the subset of patients with acute myocardial infarction. It remains to be investigated whether transient myocardial retention could account for the absence of functional effects and identify clinical non-responders to the therapy. In addition, in chronic ischaemic heart failure, no myocardial activity is detected at 20 h after the injections, making the intracoronary approach controversial for treatment of this patient population.[19]

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Competing interests: JB is a member of a non-profit organisation which is a founding member of Cardio[3].

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